DETERMINATION OF GLUCOSE, GALACTOSE, AND RHAMNOSE IN MIXTURES*

By WILLIAM L. PORTER and CHARLES S. FENSKE, Jr. (Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia 18, Pa.)†

In a study of the flavonol glycosides of buckwheat and other plants, it was desirable to determine the carbohydrates in their hydrolysates, both qualitatively and quantitatively. Chemical methods for the separation of glucose, rhamnose, and galactose (6) were not suitable because they were tedious, quantitatively inaccurate, and not successful in identifying sugars present in trace amounts. Polarimetric methods (3) for analyses of the possible combinations were not applicable because of interfering substance resulting from the hydrolysis, and also because of the limited quantities of most of the materials to be analyzed. A combination of methods—namely, filter paper chromatography (5), copper reduction (4), and yeast fermentation (1, 2, 7, 8)—was finally employed, with complete success. Although the procedure to be presented deals exclusively with mixtures of glucose, rhamnose, and galactose, the principles may be applied in analyses of mixtures of other sugars.

METHODS

Hydrolysis.—Several methods of hydrolysis were employed. However, boiling under reflux for 2 hours in 2.5 per cent sulfuric acid, or for 1 hour in 5 per cent sulfuric acid, gave the best recoveries of quercetin and sugars from rutin. Before analysis, the sulfuric acid was neutralized with sodium hydroxide.

Copper Reduction.—The sugar analyses were made by Schoorl's method (4).

Paper Chromatography.—The method of Partridge (5) was used. The solvent employed was the mixture of 40 per cent butanol, 10 per cent ethanol, and 50 per cent water, described in the original paper.

Fermentation.—A culture of the yeast *Saccharomyces bayanus* N.R.R.L.‡ No. 966) was used for fermenting glucose and one of *Saccharomyces carlsbergensis* (N.R.R.L. No. 379) was employed for fermenting glucose and galactose. The methods of fermentation used for the two yeasts were similar to those employed by Wise and Appling (8) and by Auernheimer *et al.* (2) except that the fermentation time was lengthened to 48 hours and the flasks were not shaken continuously.

---

* Presented at the Meeting-in-Miniature, Philadelphia Section, of the American Chemical Society, Philadelphia, Pa., in January 1949.

† One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

‡ Northern Regional Research Laboratory.

TABLE 1.—*Analyses of solutions of single sugars and synthetic mixtures of sugars by copper reduction and selective fermentation*[a]

SUGAR	SINGLE SUGARS			MIXTURES OF TWO SUGARS			MIXTURE OF THREE SUGARS
	GLUCOSE	GALACTOSE	RHAMNOSE	GLUCOSE+GALACTOSE	GLUCOSE+RHAMNOSE	GALACTOSE+RHAMNOSE	GLUCOSE+GALACTOSE+RHAMNOSE
Before Fermentation							
Mg. Sugar/aliq.[b]	55.20	54.76	50.00	*22.08+21.91*	*22.08+20.00*	*21.91+20.00*	*16.56+16.43+15.00*
Ml. Thio. Found	16.65	14.94	14.33	12.90	11.91	12.55	14.35
Ml. Thio. Calc.	16.69	15.11	14.33	13.13	12.17	12.84	14.39
% Recovery	99.8	98.9	100.0	98.3	97.9	97.8	99.7
After Fermentation with Yeast N.R.R.L. No. 966 to Remove Glucose Only							
Mg. Sugar/aliq.[b]	16.56	16.43	15.00	*16.56+16.43*	*16.56+15.00*	*16.43+15.00*	*9.94+9.86+9.00*
Ml. Thio. Found	0.00	4.79	4.68	4.70	4.50	9.00	5.60
Ml. Thio. Calc.	0.00	4.69	4.49	4.70	4.48	9.19	5.66
% Recovery	—	102.1	104.2	100.0	100.2	98.0	98.9
After Fermentation with Yeast N.R.R.L. No. 379 to Remove Glucose and/or Galactose							
Mg. Sugar/aliq.[b]	16.56	16.43	15.00	*16.56+16.43*	*16.56+15.00*	*16.43+15.00*	*9.94+9.86+9.00*
Ml. Thio. Found	0.00	0.00	4.60	0.00	4.33	4.38	2.68
Ml. Thio. Calc.	0.00	0.00	4.49	0.00	4.48	4.48	2.74
% Recovery	—	—	102.5	—	96.7	97.8	98.0

[a] Duplicate stock solutions of the single sugars were made to volume. Aliquots of these solutions were mixed to give the sugar mixtures. All analyses were then made in duplicate.

[b] Mg. sugar/aliquot = weight of the anhydrous sugar used in the final copper reduction; equivalent to the calculated ml. of thiosulfate.

PROCEDURE

Prepare a filter paper chromatogram of the solution to be analyzed. Determine the identity of the sugars present from the position of the spots. Dilute an aliquot of the original solution in the same manner as was done with the fermented samples. Using Schoorl's method, analyze an aliquot of this solution for total reducing sugars. Record the volume of 0.1 *N* thiosulfate required. If the chromatogram indicates that rhamnose is absent, but that either glucose or galactose or a mixture of these two is present, follow fermentation procedure A described below. If rhamnose is present in combination with either or both of the two sugars, follow procedure B. If the chromatogram indicates only rhamnose, fermentation is not necessary.

Fermentation Procedure A.—Ferment an aliquot of the solution with yeast N.R.R.L. No. 966 to remove glucose. Determine reducing materials on an aliquot of the fermented solution by Schoorl's method. Record the volume of 0.1 *N* thiosulfate. If the volume is zero, only glucose is present in the original solution. Calculate the weight of glucose present in the original solution by means of Schoorl's table from the volume of 0.1 *N* thiosulfate used before fermentation. If titration is required, the volume of 0.1 *N* thiosulfate is then equivalent to the galactose. From this volume, calculate the weight of glucose present in the original solution. If the two volumes are equal, only galactose is present in the original sample. Calculate the weight of galactose from either titration. If the two volumes are not equal, subtract the volume used by the solution after fermentation from the volume required for the total reducing sugars, to obtain the volume of 0.1 *N* thiosulfate equivalent to the glucose. Calculate the weight of glucose present in the original solution.

Fermentation Procedure B.—Ferment one aliquot of the original solution with yeast N.R.R.L. No. 966, and another with N.R.R.L. No. 379. The volume of 0.1 *N* thiosulfate after fermentation with yeast No. 379 is equivalent to the rhamnose in the aliquot taken for copper reduction, since both the glucose and/or galactose would be destroyed. Calculate the weight of rhamnose present in the original solution. The volume of 0.1 *N* thiosulfate after fermentation with yeast No. 966 is equivalent to the rhamnose and/or galactose, since only glucose would be destroyed. The difference between the volumes of 0.1 *N* thiosulfate required after fermentation with yeast No. 966 and No. 379 is equivalent to the galactose present. Calculate the weight of galactose present in the original solution. Subtract the volume of 0.1 *N* thiosulfate required after fermentation with yeast N.R.R.L. No. 966 from the volume of 0.1 *N* thiosulfate used in the aliquot analyzed before fermentation to obtain the volume of 0.1 *N* thiosulfate equivalent to the glucose. Calculate the weight of glucose in the original solution.

RESULTS

Table 1 shows the results obtained by using the above-described procedure with pure sugars, and with the several possible combinations of these sugars. To simplify the presentation of the data, the values are reported as ml. of 0.1 *N* thiosulfate rather than as mg. of sugar, since it is impossible to evaluate recoveries for any one step of the over-all procedure without incorporating any errors present in all the other steps. The experimental titres for a given weight of sugar are compared with the calculated titres. Percentage recovery is the ratio of ml. of thiosulfate found to the calculated volume of thiosulfate. Examples of an application of the method to the analysis of rutin are given in Table 2.

TABLE 2.—*Recoveries of Quercetin and Sugars from Rutin*

WEIGHT OF RUTIN	H_2SO_4		TIME OF HYDROLYSIS	RECOVERY OF THEORETICAL		
	CONC.	VOL.		QUERCETIN[a]	RHAMNOSE	GLUCOSE
g.	*per cent*	*ml.*	*hrs.*	*per cent*	*per cent*	*per cent*
0.5000	2.5	25	2	100.3	95.5	96.0
.5000	5.0	25	1	100.6	95.2	95.8

[a] Quercetin was filtered on Gooch crucible after hydrolysis, washed with water, dried to constant weight at 110°C. and weighed.

DISCUSSION

As indicated by the data, synthetic mixtures of glucose, galactose, and rhamnose can be analyzed, with recoveries of 98 to 104 per cent; hydrolysates of a flavonol glycoside can be analyzed, with recoveries of approximately 96 per cent. The value 96 per cent is probably low because of destruction of sugar during the hydrolysis. By use of the qualitative filter paper chromatogram, the amount of fermentation and chemical work required is reduced to the minimum. Application of the technique to other mixtures is being investigated.

SUMMARY

A method is presented for the analyses of mixtures of glucose, galactose and rhamnose in hydrolysates of flavonol glycosides. The sugar determinations are made by Schoorl's copper reduction method before and after fermentation by two yeasts capable of selective destruction of glucose and of glucose and galactose, respectively. Filter paper chromatography is used for qualitatively identifying the sugars.

ACKNOWLEDGMENTS

Grateful acknowledgement is made to T. C. Gordon and A. L. Everett of the Eastern Regional Research Laboratory staff for their aid in preparing the yeast suspensions and in working out the fermentation techniques; and to L. J. Wickerham of the Northern Regional Research Laboratory, Peoria, Ill., for supplying the organisms used in this investigation.

LITERATURE CITED

(1) APPLING, J. W., RATCLIFF, E. K., and WISE, L. E., *Anal. Chem.*, 19, 496 (1947).
(2) AUERNHEIMER, A. H., WICKERHAM, L. J., and SCHNIEPP, L. E., *Ibid.*, 20, 876 (1948).
(3) BROWNE, C. A., and ZERBAN, F. W., "Physical and Chemical Methods of Sugar Analysis," 3rd ed., page 981, New York, John Wiley and Sons, 1941.
(4) *Ibid.*, page 828.
(5) PARTRIDGE, S. M., *Nature*, 158, 270 (1946).
(6) PERKIN, A. G., *J. Chem. Soc.* 97, 1776 (1910).
(7) WISE, L. E., and APPLING, J. W., *Ind. Eng. Chem. Anal. Ed.*, 16, 28 (1944).
(8) *Ibid.*, 17, 182 (1945).